UDC 637.5(075.8)
DOI: 10.15587/1729-4061.2017.110987

DETERMINING THE EFFECT OF LUPIN FLOUR AND INULA ON THE FLOW OF METABOLIC PROCESSES IN THE ORGANISM

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На білих мишах проведено вивчення токсичності люпинового борошна, кореня дивосилу та функціональних котлет. Досліджено, що люпинове борошно та дивосил не викликають катарального або геморагічного запалення шлунково-кишкового тракту та загибель мишей, отож є нетоксичні. При згодовуванні мишам котлет з 10 % вмістом люпинового борошна та 0,5 % дивосилу встановлено, що при патологоанатомічному розтині макроскопічних змін в органах і тканинах не виявлено. Маса органів ($p<0,05$) є в межах норми та підтверджує, що даний продукт можна включати в раціон харчування людей

Ключові слова: м'ясний фарш, люпинове борошно, дивосил, функціональні котлети, раціональне харчування, патологоанатомічний розтин, обмінні процеси, посічені напівфабрикати

На белых мышах проведено изучение токсичности люпиновой муки, корня девясила и функциональных котлет. Доказано, что люпиновая мука и девясил не вызывают катарального или геморрагического воспаления желудочно-кишечного тракта и гибель мышей, поэтому являются нетоксичными. При скармливании мышам котлет с 10 % содержанием люпиновой муки и 0,5 % девясила установлено, что при патологоанатомическом вскрытии макроскопических изменений в органах и тканях не обнаружено. Масса органов ($p<0,05$) находится в пределах нормы и подтверждает, что данный продукт можно включать в рацион питания людей

Ключевые слова: мясной фарш, люпиновая мука, девясил, функциональные котлеты, рациональное питание, патологоанатомическое вскрытие, обменные процессы, секущиеся полуфабрикаты

1. Introduction

Functional minced meat semi-finished products harmoniously combine high gustatory qualities, nutritional value with positive functional properties and produce a positive impact on human health. They are at the same time intended for a wide range of consumers and can be used regularly as a part of a normal diet without any specific recommendations.

At the World Congress in the USA on the problems of using vegetable proteins for food and feed purposes, lupin was described as an important reserve of high quality proteins [1]. The use of lupin seeds in food industry is limited because of the existence of bitter and poisonous alkaloids in it. However, at present, such varieties of white lupin as "Kharchovyi", "Synii parus", "Olezhka", "Volodia", "Volodymir" have been cultivated. The main feature of it is that its proteins do not require thermal treatment, as they do not contain inhibitors of proteolytic enzymes: trypsin and chymotrypsin, phytohemoglutenins, neurotoxins and alpha-galactoses [2].

Food lupin belongs to organic crops because it is grown without chemical fertilizers. Unlike other legumes, seeds of white food lupin contain 10...12 % of fat, a complex of vitamins, macro-and microelements and other bioactive substances. They protect the organism from radionuclides and heavy metals, as well as accelerate the process of their removal. Dietary fibers of the given varieties of lupin, which are mainly contained in the shell (80...88 %) with the rest in the nucleus of the seed (15....18 %), are pretty good enterosorbents of radionuclides, cesium, strontium and other heavy metals [3]. The effect of enterosorption is revealed also in relation to cholesterol, bile and other metabolism products. Thus, the grain of food varieties of white lupin should be used in the production of high-protein foods for children and for dietary and medicinal-prophylactic purposes.

Inulin (to 44 %), inulenin, pseudo inulin, acetic and benzoic acids were found in the root of inula [4]. Inulin is known to be able to withdraw radionuclides and heavy metals form the body, to stimulate the growth of bone tissue, absorption

of calcium in the human body [3, 4]. That is why development of the minced meat semi-finished products with the content of lupin and inula is relevant today.

Therefore, testing the products for toxicity on the organisms of white mice is necessary in order to include this product in the diet of humans.

2. Literature review and problem statement

Under modern conditions, the agricultural complex of Ukraine faces the challenge of increasing production of food products and improving their quality and safety. Today, in accordance with the requirements of the international system of self-control (HACCP), the problem of quality control and food safety is becoming increasingly important [5, 6].

A great number of scientists explore the issues of nanocitrates, comparing toxicity of analogues [7–12].

There are known developments of production of minced meat semi-finished products, which include introduction of meat extracts and powders, by-products or products of their processing, hydrated soy protein, vegetable and dietary supplements, dietary fibers, oil, etc. into mince. Shortcomings of these developments include complexity of formulation of preparation, unavailability of ingredients, low food and biological value of produce. In particular, papers [8–11] focus on the introduction of minced semi-finished products of protein components, mainly soy concentrates, bran, whey, etc. to meat.

The issue of control of the content of heavy metals in meat produce is paid special attention to. Concentrations of elements of aluminum, arsenic, cadmium, chromium, cobalt, copper, lead, mercury, nickel, selenium and zinc get into meat during air and vegetation contamination [12]. In particular, according to results of examination of meat of wild and domestic animals [13], it was found that the existence of these elements does not pose any danger to the health of consumers, their amount does not exceed maximum permissible concentrations [14]. The level of accumulation of particular heavy metals was determined in meat and liver of sheep. According to the examination of the longest back muscle and liver samples, it was found that as the age of sheep increases, the content of water in meat and the content of protein, fat and ash increases, and the accumulation of Cd and Pb depends on the age of animals [15].

In Serbia and Spain, toxicity of food is examined by determining the content of Pb, Cd, Hg, As. The existence of these elements in products was found to result in a daily consumption of 72.30 μg of them, 21.89 μg of As and 11.51 μg of Cd by an adult person [16].

Examination of heavy metals in sausage products is not less important, in particular, control of dry fermented sausages is performed in Lisbon (Portugal). Results of the studies found that heavy metals exist in sausages, but they do not cause danger to consumers [17].

The most effective method of testing product quality is conducting research on white mice [18, 19].

Thus, based on a critical analysis of the sources, it was found that the studies that were carried out in Lisbon, Serbia and Spain focused on determining the content of toxic substances and heavy metals. It is necessary to reduce ways of contamination of raw materials. Development of new formulations was not perfect, that is why the products were of low nutritional value and quality. Determining the toxicity on living organisms is the most effective method for testing quality and the introduction of raw materials to production.

3. The aim and objectives of the study

The aim of present research is to determine toxicity of lupin flour and inula root and meat cutlets on white mice, in order to introduce functional cutlets to the market and in the diet of people.

To accomplish the set aim, the following tasks had to be solved:

– to determine toxicity of lupin flour, inula, functional cutlets that contain 5 %, 10 %, 15 % of lupin flour with a replacement of meat share and 0.5 % of inula, under conditions of the intragastrical introduction of 0.5 ml of the extract;

– to determine toxicity of the finished 10 % functional cutlets, under conditions of feeding them as the main feed to white mice for 10 days;

– to carry out toxicity analysis on the pathoanatomical dissection of mice, examining the condition of internal organs and to perform a hematological analysis of blood.

4. Materials and methods for toxicological and hematological research

We applied appropriate methods, which were used at the Laboratory of Pharmacology and Toxicology and the Laboratory of Clinical-Biological studies of the State Scientific-Research Control Institute of Veterinary Preparations and Feed Additives (Lviv, Ukraine).

The experiments for studying toxicity of raw materials and products were carried out on white mice (Fig. 1). They involved appropriate methods, under conditions of intragastrical introduction and feeding on cutlets with 10 % of lupin flour and 0.5 % of inula for 10 days.

Fig. 1. Laboratory white mice

The methods for determining toxicity under conditions of intragastrical introduction of the extract, determining toxicity of the finished 10 % cutlets under conditions of feeding them to white mice as the main feed for 10 days, hematologic research, as well as the formulation and the technology of preparation of functional cutlets with lupin flour and inula are presented in paper [20].

5. Results of toxicological and hematological research

5. 1. Results of research into determining the toxicity of lupin flour, inula and functional cutlets

The death of white mice within three days was not observed.

During the pathoanatomical dissection of animals, put to sleep by chloroform, which were given lupin flour, it was found: fur cover was bright, smooth, there was no damage, natural openings were closed, and there was no secretion; during internal examination, hypodermic fat tissue was found to be well developed (Fig. 2).

Fig. 2. Examination of mice before pathoanatomical dissection

Position of the internal organs of the chest and abdominal cavities was anatomically correct. The liver was a of dark cherry color, with sharp edges, of elastic consistency with separate blazes of light color. The spleen is of regular shape, dark-red, with sharp edges and characteristic structures on the cut, not enlarged. The kidneys were of bean-like shape, there was a fat capsule, of dark cherry color, elastic consistency, not enlarged, the boundary between the cortical and medullar areas was preserved. The lungs had friable consistency, were pale pink, without any obvious microscopic changes. The heart was of cone-like shape, myocardium was dark red, elastic. The pancreas was pale pink, of lobar structure, without any obvious macroscopic changes. The stomach was filled with feed masses, the mucous membrane is light pink, smooth, bright, slightly dry. The small and large intestines are unevenly filled with feed masses, the mucous membrane is smooth, bright, and wet.

During the pathoanatomical dissection, in animals, put to sleep by chloroform, which were given inula, it was found that fur cover was bright, smooth, with no damage, natural openings were closed, there was no secretion. The position of the internal organs of the chest and abdominal cavities was anatomically correct. The liver was of dark cherry color, with sharp edges. The spleen was of regular shape, of dark red color, with the sharp edges, of characteristic structure at the cut, not enlarged. The kidneys are of bean-like shape, with fat capsule, of dark cherry color, of elastic consistency, not enlarged, the boundary between the cork and brain zones was preserved. The lungs had friable consistency, pale pink.

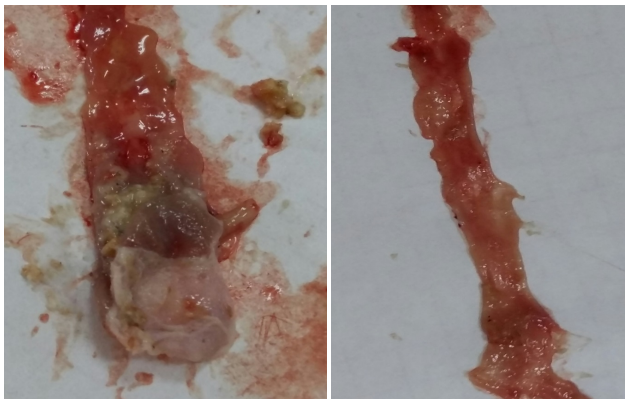
The heart was of cone-like shape, the myocardium was dark red, elastic. The pancreas was pale pink, with lobar structure, without any apparent macroscopic changes. The stomach was moderately filled with feed masses. Small intestine was filled with feed masses, the loops of the intestine were homogeneous, without bloating, mesentery vessels are hardly visualized. Large intestine is filled with fecal masses of characteristic shape and consistency. The mucous membrane of the stomach, large and small intestines was smooth, bright, wet, without layering (Fig. 3).

Fig. 3. Pathoanatomical dissection. View of internal organs

During the pathoanatomical dissection, in animals, put to sleep by chloroform, which were given the extract of cutlets with 5 % of lupin flour and 0.5 % of inula, it was found that there were no changes in the fur cover, visible mucous membranes and natural openings. The position of the internal organs of the chest and the abdominal cavities is anatomically correct. The liver is homogeneous, of dark red color, with sharp edges, with characteristic structure at the cut. The spleen is with sharp-edged, of dark cherry color, homogeneous, the characteristic structure at the cut is preserved, the edges of the cut converge. The kidneys are bean-shaped, there is a fat capsule, of dark cherry color, of elastic consistency, not enlarged, the boundary between the cork and brain zones is preserved. The lungs have loose consistency, pale pink color, without any apparent macroscopic changes. The heart was of cone-like shape, the myocardium was dark red, elastic. The pancreas was pale pink, with lobar structure, without any apparent macroscopic changes. The stomach was moderately filled with feed masses, the mucous membrane was without apparent changes, the small department of intestine is moderately filled, the contents were homogeneous. The content of large intestine had paste-like consistency, with characteristic smell. The mucous membrane of the thin and large intestine was pale pink, bright, moist, without layering.

During pathoanatomical dissection, in animals, put to sleep by chloroform, which were given the extract of cutlets with 10 % of lupin flour and 0.5 % of inula, it was found: the position of the internal organs of the chest and the abdominal cavities was anatomically correct. Venous congestion was observed in parenchymatous organs. The liver was homogeneous, dark red, with sharp edges, of characteristic structure at the cut. The spleen was sharp-edged, of dark cherry color, homogeneous, of characteristic structure at the cut, the edges of the cut converged. The kidneys were bean-shaped, there was a fat capsule, of dark cherry color, of elastic consistency, not enlarged, the boundary between the corti-

cal and medullar zones was preserved. The lungs had friable consistency, pale pink, without changes. The heart was of a cone-like shape, the myocardium was dark red, elastic. The pancreas was pale pink, with lobar structure, without any apparent macroscopic changes; the stomach was of regular shape, the loops of intestines were evenly located, without inflation, moderately filled with contents. The mucous membrane of the small and large intestine was pale pink, smooth, bright, and moist (Fig. 4).



a *b*

Fig. 4. View of mucosa: *a* — stomach; *b* — intestines

During pathoanatomical dissection, in animals, put to sleep by chloroform, which were given the extract of cutlets with 15 % of lupin flour and 0.5 % of inula, it was found that fur cover was bright, smooth, without any damage, natural openings were closed, without secretion. During external examination it was found that the position of the internal organs of the chest and abdominal cavities were anatomically correct. Congestion of mesentery vessels was observed (Fig. 5).

Fig. 5. Congestion of mesentery vessels

The liver is of elastic consistency, homogeneous, of dark red color, with sharp edges, with characteristic structure at the cut. The spleen was sharp-edged, of dark cherry color, homogeneous, of the characteristic structure at the cut. The kidneys were bean-shaped, there was a fat capsule, of dark cherry color, of elastic consistency, not enlarged, the boundary between the cork and brain zones was preserved. The lungs had friable consistency, of pale pink color. The heart was of cone-like shape, the myocardium was dark red, elastic. The pancreas was pale pink, with lobar structure, without any apparent macroscopic changes. The stomach was well filled. Small intestine was filled with feed masses. The rectum contained formed fecal masses of characteristic form, there were no fermentation processes. The mucous membrane of the small and large intestine was pale pink, smooth, bright, moist, without layering.

5. 2. Results of research into determining the toxicity of 10 % cutlets under conditions of feeding

In the course of research, no changes in behavior of mice, which were on a standard diet (control group), and in the animals that received the finished cutlets, were noticed, the animals were active, with no signs of external aggression. Preservation of animal was at 100 % level. During pathoanatomical dissection, no macroscopic changes in the organs and tissues were found (Fig. 6).

Fig. 6. Pathoanatomical dissection of mice of experimental group, fed on cutlets

Table 1 gives an analysis of the internal organs of mice from control and experimental group.

Table 1

Analysis of internal organs

Group		Liver	Lungs	Heart	Spleen	Kidneys	Body weight
Control	1	0.92	0.50	0.07	0.09	0.32	22.2
	2	0.99	0.38	0.09	0.12	0.34	21.8
	3	0.92	0.28	0.11	0.19	0.36	19.5
M±m		0.94±0.02	0.38±0.07	0.09±0.01	0.13±0.03	0.34±0.01	21.17±0.84
Experimental	4	1.17	0.24	0.13	0.19	0.38	23.0
	5	1.04	0.21	0.10	0.15	0.25	20.0
	6	1.38	0.30	0.13	0.20	0.31	21.7
M±m		1.2±0.1	0.25±0.03	0.12±0.01	0.18±0.02	0.31±0.04	21.57±0.87
P		p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

In all internal organs the weight changes are $p<0.05$, which is within the normal range. This indicates that this produce is not toxic.

5. 3. Results of hematologic research

Hematologic studies were conducted in the laboratory of clinical and biological research of SSRCI of veterinary drugs and feed additives. Research results are presented in Table 2–4, where No. 1–3 are the samples of the control group, No. 4–6 are the samples of the experimental group of mice. Results are within the normal range.

Table 2

Morphological parameters of blood of mice

No. of entry of sample	Erithrocytes, T/l	Hemoglobin, g/l	Hematocrit, %	MCH, Pg	MCHC, g/dl	MCV, mkm^3	Thrombocytes, g/l
1	8.2	147	41	17.8	35.2	50.7	669
2	7.2	193	34	26.8	57.1	46.9	442
3	7.8	126	37	16.0	33.8	47.4	794
4	7.8	137	38	17.1	36.0	47.7	538
5	9.1	143	40	15.6	35.5	44.1	971
6	7.3	118	35	16.1	33.5	48.2	914

Table 3

Contents of leukocytes and blood leukogram of mice

No. of entry of sample	Leukocytes, g/l	Leukogram, %		
		Limphocytes, %	Monocytes, %	Granulocytes, %
1	8.4	69.8	10.5	19.7
2	8.2	64.7	9.0	26.3
3	7.8	71.0	11.0	18.0
4	8.9	66.3	11.2	22.5
5	5.9	75.7	6.1	18.2
6	5.8	76.2	13.2	10.6

Table 4

Biochemical indices of blood serum of mice

No. of entry of sample	Crude protein, g/l	Creatinin, mkmol/l	Urea, mkmol/l	AST, unit/l	ALT, unit/l	ALP, unit/l	Alpha-amilase, unit/l	Glucose, mkmol/l
1	6.07	46.9	7.6	243.2	52.6	663.5	1652	5.40
2	6.45	48.5	6.3	237.6	48.6	258.6	2113	5.16
3	7.10	42.7	8.5	227.7	55.1	443.9	1821	4.67
4	6.02	37.7	8.8	255.5	61.1	479.5	2075	4.47
5	5.25	47.7	8.5	253.1	52.3	385.1	1781	5.08
6	5.47	41.8	7.9	275.1	49.7	516.6	1943	6.29

Results of hematological research after a 10-day feeding of cutlets were in the normal range. Therefore, this product has no negative influence on the organism of mice. According to "Methods for determining toxicity on laboratory animals", raw materials and cutlets can be considered non-toxic.

6. Discussion of results of determining the toxicity of produce

Food products must meet the requirements of State standards, technical conditions, including safety indicators. The developed meat minced semi-finished products form beef with addition of lupin flour and inula were studied using the methods for determining toxicity that were used in the State scientific research institute of control of veterinary preparations and feed additives. We also used "Methods for determining toxicity of oil-seed meal, cakes and fodder yeast" and "Methods for determining toxicity on laboratory animals" on the organism of white mice weighing 20–23 g.

Tasting of functional cutlets enabled us to choose the best sample (No. 2, containing 10 % of lupin flour and 0.5 % of inula), which is being patented and tested in order to be introduced into production. The usage of these methods made it possible to prove non-toxicity of the developed product and its composition.

Developed functional cutlets can be included in the diet to solve problems of protein deficiency. The shortcoming of the development is that in Ukraine there is no effective production of lupin flour, compared with Poland. In fact, lupin flour is a source of valuable protein, which can be widely applied in combination with different products. For example, in Poland there is organized production of bread and pasta.

Functional cutlets with replacement of meat share with lupin flour will also be economically more profitable. Results of this research can be implemented in industrial production of sections of minced semi-finished products. Beef cutlets with lupin can be widely used in fast food, and thereby change the stereotypes about harmfulness of fast-food ("street food"). Possible directions of development of this study will be extension of the range of meat products with the lupin share, in particular, production of sausage, sausages, dumplings and khinkali with meat-lupin mince.

7. Conclusions

1. It was found that lupin flour, inula root and functional cutlets containing 5 %, 10 %, 15 % of lupin flour with replacement of meat share and 0.5 % of inula as a spicy-aromatic additive do not cause catarrhal or hemorrhagic inflammation of the gastrointestinal tract and the death of mice, so they are non-toxic.

2. The mice were fed on cutlets with 10 % content of lupin flour and 0.5 % of inula. It was found that during pathoanatomical dissection, macroscopic changes in the organs and tissues were not found, $p<0.05$, which is in the normal range.

3. The authors obtained results of hematologic research in mice blood, in particular, morphological parameters, contents of leukocytes and leukogram, biochemical indices, which prove that this product can be included in the diet of humans.

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